

**Mosquitofish avoid thermal stress by moving from open water to the shade of the mangrove *Rhizophora mangle***

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Short title: Mosquitofish avoid thermal stress using mangrove shade

**ABSTRACT:** Mangrove trees provide environmental buffering for animals by reducing day time water thermal maxima. Shade from *Rhizophora mangle* trees reduces thermal stress for the mosquitofish, *Gambusia affinis*. Data were collected from mangrove forests in Quintana Roo, Mexico. Soliman Bay, a mangrove forest decoupled from direct ocean water, and La Lunita with semi-direct access to ocean water. During cooler morning hours at Soliman Bay large numbers of mosquitofish foraged in open channels. At the same time, few mosquitofish utilised shaded areas within the *R. mangle* prop roots. When channels exceeded water temperatures above 38°C mosquitofish migrated into the shaded areas provided by *R. mangle*

trees. Channel water reached a daytime maximum temperature of 46°C, while daytime shaded *R. mangle* areas remained 6.2°C cooler. Temperature rise, and abundance shifts were not found in La Lunita, which has water temperatures of 27 to 28°C throughout the day. Size distributions between both localities showed mosquitofish caught in Soliman Bay to be more than 10 per cent smaller than the mosquitofish from La Lunita, which had a higher abundance of mature adults. In Soliman Bay mosquitofish were small, and the thermal stresses may impose serious developmental impacts that would be reduced by occupying water shaded by *R. mangle*. *Rhizophora mangle* provide a refuge of environmental buffering for fish and future increasing water temperatures may reveal a greater use of this little-known mechanism. This study provides an example of a mechanism whereby mangroves support intertidal biodiversity through environmental buffering.

KEY WORDS: Biodiversity mechanism, mangroves, thermal tolerance, fish autecology

## 1. INTRODUCTION

Mangrove forests are important marginal habitats that provide physical and environmental protection for the resident fauna (Laegdsgaard & Johnson 2001, Hendy et al. 2014). They provide a range of ecosystem services such as erosion reduction, carbon sequestration and biodiversity maintenance (Donato et al. 2011, Hendy et al. 2014, Duke & Schmitt 2015, Eddy et al. 2016). These ecosystem services are being reduced due to the loss of mangrove forests, with increasing ecological and economic consequences for the fauna and people who rely on them (Alongi 2002, Duke et al. 2007).

Impacts from altering the mangrove hydrology by building roads across mangrove habitats has caused some mangrove systems to become decoupled or semi-decoupled, depending on whether connection to open ocean water has been completely or partially lost

(Teutli-Hernández & Herrera-Silveira 2018). These same impacts have been imposed on the mangrove sites in this present study. The loss of connection may lead to reduced biodiversity due to raised water temperatures and reduced dissolved oxygen (Lewis et al. 2016).

Over the last 30 years increases of the sea surface temperatures (SST) have been observed, with estimates of an average increase of almost 4 °C by the year 2100 (Aral & Guan 2016, Maulvault et al. 2017). Increases of SST may lead to behavioural, physiological and developmental problems for marine fish (Pershing et al. 2015, Jeffries et al. 2016, Maulvault et al. 2017).. The rapid warming in the Gulf of Maine led to a decline in Atlantic cod (*Gadus morhua*) recruitment due to an increase in heat-induced mortalities (Pershing et al. 2015). Californian longfin smelt suffer cellular stress due to an upregulation of heat shock proteins when exposed to temperatures beyond their zone of tolerance (Jeffries et al. 2016) and a range of estuarine fish have been shown to suffer from oxidative stress when thermally impacted (Madeira et al. 2013). Thus, with increasing concern for future fisheries, the inability of marine fauna to adapt or survive to rising temperatures will create significant impacts from species- to population-levels and global scales (Pörtner & Knust 2007). Distributions of some pelagic fish species are migrating poleward to cope with SST rise (Donet et al. 2011, Pershing et al. 2015). Lack of thermal adaptation may be important to tropical species living within narrow thermal ranges (Munday et al. 2017). Coupled with losses of habitat complexity and ecosystem services, the continued loss of mangrove forests (Duke et al. 2007, Teutli-Hernández & Herrera-Silveira 2018) may see dramatic declines of terrestrial fauna (Rog et al. 2016) and fish (Muzaki et al. 2017) leading to declines of future fauna biomass for mangroves and adjacent fisheries. The loss of species and populations driven by a warming climate is a global concern (Wiens 2016). Critical thermal limits can deplete genetic diversity, have cascading effects on other species, facilitate trophic collapse, and alter ecosystem functioning and services (Luck et al. 2003, IPCC 2014). Critical thermal

limits will impact many organisms, particularly in the marine environment (Pinsky et al. 2019).

A key mangrove ecosystem service is its nursery function (Laegdsgaard & Johnson 2001, Lee et al. 2014, Duke & Schmitt 2015, Muzaki et al. 2017), which benefits reef fish populations by enhancing development and maturation of juveniles within the complexity of root systems that provide shelter from predators (Laegdsgaard & Johnson 2001, Hendy et al. 2013). Mangrove roots also buffer wave energy (Ismail et al. 2012) and oxygenate the sediments (Scholander et al. 1955). But little is known of other key biodiversity maintenance mechanisms in mangrove forests, particularly from environmental buffering. Hendy et al. (2013 & 2014) found a little-known biodiversity mechanism inside fallen wood within Indonesian mangrove forests. They showed that evaporative cooling within abandoned teredinid tunnels in wood was shown to benefit a wide range of terrestrial and aquatic juvenile animals. The environmental buffering delivered by cooling wood benefit many juvenile and vulnerable animals including heat-tolerant fish, octopods, spiders, crabs and polychaetes (Hendy et al. 2013 & 2014) and temperatures inside tunnelled wood were 14°C cooler than ambient temperatures (Hendy et al. 2013). In this study we report another example of behaviour that exploits the cooler parts of the mangrove ecosystem: mosquitofish move to the shade of *Rhizophora* trees when temperatures in open water reach stressful levels.

The mosquitofish (*Gambusia affinis*) is a brackish-water tolerant poeciliid fish possessing a native range that stretches across the USA, Mexico and parts of the Caribbean (Lee et al. 2017). Introduced to some temperate regions as a biological control against mosquito larvae, this viviparous fish now has a global distribution (Lee et al. 2017). Known for expressing high phenotypic plasticity, this adaptable fish has been used across a broad range of ecological studies (Winkler 1979, Wurtsbaugh & Cech 1983, Benoît et al. 2000) and

is known for its ability to thrive in extreme environmental conditions such as low dissolved oxygen, high salinities and high temperatures (Wurtsbaugh & Cech 1983). Organisms will have limits to tolerance of extremes of each of these environmental variables. In the case of water temperature, the limits are expressed as critical temperature, above which the organisms start to exhibit a loss of equilibrium and become moribund (Hupfeld et al. 2015).

In this study we investigated how shade from the canopy of *Rhizophora mangle* may offer environmental buffering for resident mosquitofish. Observations were made in two Caribbean mangrove forests on the south-western coast of the Yukatan Peninsula (Fig.1), one (Soliman Bay) with limited water exchange and consequently marked temperature fluctuation and the other (La Lunita) with a semi-direct connection to the local microtidal regime and thus much more buffered water conditions. These forests both maintain populations of *Gambusia*, but under distinctly different regimes of solar illumination and temperature fluctuation.

We hypothesised that within *R. mangle* stands:

1. Water temperature is a key diurnally-varying environmental variable for mosquitofish behaviour
2. Mosquitofish avoid dangerous water temperatures by shade-seeking behaviour
3. Light levels and food availability affect mosquitofish distribution
4. The combination of diurnally fluctuating environmental factors in the more environmentally-stressing study site will affect mosquitofish population size distribution

The objectives of this study were to:

1. Assess mosquitofish abundance in open water channels and in the shade of *R. mangle* trees throughout the day across two mangrove systems

2. Characterise behaviour of mosquitofish and evaluate the significance of this behaviour for fish autecology
3. Assess food availability in both mangrove forests at morning and afternoon hours using plankton tows
4. Quantify mosquitofish size distributions across two mangrove forests

## 2. MATERIALS AND METHODS

### 2.1. Sites used in the investigation

Observations of fish behaviour were made in two *Rhizophora mangle*-dominated mangrove forests in Quintana Roo, Mexico. The Soliman Bay dwarf mangrove forest (Fig. 1, 380 ha, 20°16'44.18'N, 87°22'55.32'W) has a canopy height generally less than 1.5m, but at the strandline, which was identified by the accumulation of buoyant plant detritus at the upper limit of tidal reach, has stands of *Conocarpus erectus*, *Laguncularia racemosa* and *Avicennia germinans*. From the strandline mono-specific stands of dwarf *R. mangle* extend across the whole 380 hectares of mangrove forest area. which is only connected to the sea via seepage through a sand bar and possibly via subterranean cenote connections. The other site is a higher canopied mangrove forest at La Lunita (Fig. 1, 2.9 ha, 20°24'26.5'N, 87°18'27.5'W), which has connections to the sea via a tidal creek that enters Yal Kul lagoon and via cenote-connected subterranean channels.

The lack of a channel connecting the Soliman Bay mangrove forest with the sea means that the fish population there is usually unable to move beyond the forest. The contained water there is micro-tidal ( $\pm 0.5\text{m}$ ) (Teutli-Hernández & Herrera-Silveira 2018) with limited water flow and input via seawater permeating the sand bar and from rainwater, resulting in brackish water, ranging from 10 to 17 practical salinity units (psu). A channel runs through the forest with water depth ranging from 15cm to 40cm. Water temperature can

reach up to 46°C and fluctuate by up to 20°C in a 24-hour period. Dissolved oxygen (DO<sub>2</sub>) is low.

During morning hours in the Soliman Bay mangrove, channels were populated by large numbers of mosquitofish, *Gambusia affinis* Baird & Girard, 1853 (Fig. 2) which were observed foraging for food. During afternoon hours almost all fish had vanished from those channels. We believe this sharp shift in behaviour is prompted by an extreme temperature change and that mosquitofish populations in the Soliman Bay mangrove forest seek the shade offered by *R. mangle* stands to avoid thermal stress.

At the La Lunita mangrove forest strandline in the Yal Ku lagoon, *Conocarpus erectus*, *Laguncularia racemosa*, *Avicennia germinans*, and *R. mangle* trees were found, but most of the forest consists of *R. mangle* reaching heights of up to 20 metres (Fig. 3). The Yal Ku lagoon has a semi-decoupled water system with subterranean cenote connections (Gabriel et al. 2008). The tidal regime is also microtidal. Cenote water in La Lunita is deep, reaching approximately four to five metres. Salinity was low, ranging from four to six psu. Water temperature was consistent throughout the day, ranging from 27 to 28 °C. A cut path that on the landward side of the strandline of the forest gave access to cenote openings above which mosquitofish were sampled to determine the population size distribution. This path was also used for observing fish behavior and measuring water temperature.

The semi-open system of La Lunita means that aquatic animals are less restricted in their ability to migrate out of the forest and the freer water circulation. Consequently, the environmental pressures found in Soliman bay are not replicated in La Lunita. Large numbers of mosquitofish were also found here.

## **2.2. Behaviour and water variables measurements**

At Soliman Bay, mosquitofish first occurred at 20 metres from the strand line, and their abundance was estimated in open channels and in shaded areas of the *R. mangle* stands

at stations between 20 and 200 meters from the strandline. Surveys were conducted during morning (08:00 to 10:00) and afternoon (13:00 to 15:00) hours using 1 m<sup>2</sup> quadrats every three to five metres along transect lines. After positioning quadrats, a five-minute period was left to pass prior to estimating fish numbers, allowing fish to acclimatise to the disturbance. Measurements were then made every 10 minutes.

In La Lunita, estimates of mosquitofish abundances were made in the open channel and in areas of the *R. mangle* prop-root stands at 0 to 70 metres from the strandline. Surveys were also conducted during morning (8:00 to 10:00) and afternoon (13:00 to 15:00) hours every three to five metres along the transect lines. Due to practical constraints of water depth estimates of mosquitofish abundance per m<sup>2</sup> were made by observing 1m<sup>2</sup> areas of water surface.

Water temperatures at Soliman Bay were measured at three stations placed at 50, 100 and 150 metres from the strandline. At each station measurements were made in the open water and in the shade of the *R. mangle* canopy ( $\pm 0.5^{\circ}\text{C}$ , 30-minute intervals). A total of six HOBO Pendant® Temperature/Light 64K Data Loggers were deployed for these measurements.

Water temperature recordings at La Lunita were also made in open water (exposed) and shaded areas, using LCD digital thermometers with submersible probes (<https://www.testco.co.uk>). Recordings were taken every five-metres from the strandline out to 70m over a six-day period in the morning (08:00 to 10:00) and afternoon (13:00 to 15:00) hours.

### ***2.3. Fish behaviour in shaded and unshaded areas***

Mosquitofish abundance was monitored at three stations in Soliman Bay from 10:30 hrs. Over a six-day period, observations were made at 50, 100 and 150 metres along the strandline. Each day before recordings took place a 10-minute interval was left to allow for



any disturbed fish to acclimatise and for any disturbed sediment to settle. To establish mosquitofish distributions in the mangrove forests water temperature (°C) in open channels was measured with an LCD digital thermometer with submersible probe and mosquitofish abundance (per m<sup>2</sup>) and in-channel water temperatures were concurrently recorded. Water temperature measurements and fish observations were recorded over a four-and-a-half-hour period or until channel water temperature failed to increase for two or more readings.

To establish whether mosquitofish at Soliman Bay were actively seeking cooler water provided by the *R. mangle* shade or simply seeking ‘dark’ cover, we positioned three parasols (<https://www.ikea-parasol>) at 50, 100 and 150 metres from the strandline in the open water channel during afternoon hours (12:00 to 14:30). Parasols were secured by driving their poles into channel sediment. Mosquitofish abundance was recorded every 10-minutes in the shade of the adjacent *R. mangle* prop-stands and in the cover of the parasol shaded area. Concurrent water temperatures measurements were also taken from within the open channel and in the cover of the parasol shaded areas.

Salinity was measured in practical salinity units (psu) using a Bellingham and Stanley refractometer (<https://refractometershop.com/>) and dissolved oxygen was measured using a Tetra Oxygen Testing kit (<https://www.tetra.net/en/en>) measuring in milligrams per litre (DO<sub>2</sub> per mg/L). Concentrations of psu and DO<sub>2</sub> were measured at 10 locations in Soliman Bay situated every 20 metres from the strandline and 10 locations in La Lunita every seven metres from the strandline.

#### **2.4. Food Availability**

Mosquitofish diets contain large concentrations of zooplankton (Hurlbert & Mulla 1981). Crivelli & Boy (1987) found that some mosquitofish stomach contents consisted of more than 80 per cent of planktonic crustaceans. Thus, plankton tows were used to assess food availability within open water channels at both localities during morning and afternoon hours.

The plankton net had a frame diameter of 300 mm, net length of 880 mm and mesh filter size of 250  $\mu\text{m}$ . Tow-lines were pulled for five-metres in open water channels at three locations from the strandline (Soliman Bay: 50, 100 and 150 metres and La Lunita: 0, 35 and 70 metres). In each locality  $\sim 1 \text{ m}^3$  of water was sampled for plankton diversity and abundance. A total of 6 samples were collected from each location and stored in 100 ml vials with water from the study site. Each sample was gently shaken, and 10 ml of sample water was extracted from each 100 ml vial using a Stempel pipette and analysed. Numbers were extrapolated to represent abundance of plankton per  $1 \text{ m}^3$  of mangrove water between localities. Planktonic communities in the samples were examined at a magnification of x40 using a stereo microscope.

## 2.5. Size Distribution

The seine-net haul technique was used in random locations from the strandline and further out at both localities to determine mosquitofish size distributions ( $\pm 0.1 \text{ mm}$ ; standard length from the anterior tip to the caudal peduncle). The seine-nets used had a mesh size smaller than the diameters of juvenile mosquitofish. Four seine-net hauls were undertaken to capture mosquitofish at Soliman Bay, and five seine-net hauls were undertaken to capture mosquitofish at La Lunita. Photographs of each of the hauls were taken using a Nikon Coolpix A100 (<https://store.nikon.co.uk/>). Images were analysed using the digital analysis package ImageJ. Pooled sample sizes of 391 fish (391 fish were caught at one site and 391 were selected at random from the other site) from each study site were used to distinguish mosquitofish size distributions (<https://imagej.nih.gov/ij/>).

## 2.6. Statistical analyses

Temporal and spatial differences of fish distributions, and temperature ( $^{\circ}\text{C}$ ) differences from the quadrat data were examined using a General Linear Model (GLM) with time of day and

habitat (between roots or out in open channel) used as factors. Differences of temperature and fish abundances in Soliman Bay were examined using a GLM, with distance from the strandline and time as factors between sites and parasol cover compared to open channels. Paired t-tests were used to examine differences of temperatures measured in the open channels with temperatures measured within the shade of the *R. mangle* trees; mean morning and afternoon temperatures and mean morning and afternoon fish abundance in both study locations; and, dissolved O<sub>2</sub> and salinity measurements on the dimensionless practical salinity scale from both study locations. Two-Way Analysis of Variance (ANOVA) was used to analyse variations in food availability in both study locations during morning and afternoon hours with site and time of day (morning and afternoon) as factors. A one-way ANOVA was also used to examine relationships before and after temperatures reach their critical thermal limit for the mosquitofish in the Soliman Bay mangrove open-water channels and shaded areas. All count data were square root transformed and all measurement data were Log transformed with suitability of the transformations scrutinized by examining residuals. Post-hoc, Descriptive Statistics and Tukey's pairwise comparison tests were used to separate values into statistically distinct subsets in all ANOVAs. All statistical analyses were performed using MINITAB (MINITAB Inc, version 13.20).

### 3. RESULTS

#### 3.1. Fish Abundance

During morning hours in Soliman Bay the highest abundance of mosquitofish were found in the open water channel. In the afternoon, mosquitofish abundances were significantly greater in the shade amongst the roots of the *R. mangle* trees (Fig. 4 A: GLM, area (channel & roots) and time (am & pm) vs. fish abundance: area vs. fish abundance:  $F_{1,87} = 8.5$ ,  $p \leq 0.01$ ; time vs. fish abundance:  $F_{1,87} = 39.1$ ,  $p \leq 0.001$ ; and area\*time vs. fish abundance:  $F_{1,87} = 177.2$ ,  $p \leq 0.001$ ). Water temperature (°C) in the open channel was found to be significantly warmer

during the afternoon (12:00 to 18:00) when compared to water temperatures recorded from within the adjacent *R. mangle* tree shaded areas at the same time.

Water temperatures in Soliman Bay during afternoon hours were on average  $> 6^{\circ}\text{C}$  cooler in the *R. mangle* tree stands, than in open water channel temperatures. Afternoon water temperature measurements within *R. mangle* shaded areas were only  $1.7^{\circ}\text{C}$  warmer than temperatures measured in the adjacent channels during morning hours (06:00 to 12:00) (Fig. 4 B: GLM, area vs. temperature:  $F_{1,87} = 799.2, p \leq 0.001$ ; time vs. temperature:  $F_{1,87} = 606.2, p \leq 0.001$ ; and area\*time vs. temperature:  $F_{1,87} = 28.5, p \leq 0.001$ ).

Mosquitofish abundance in La Lunita remained high in open water channels throughout both morning and afternoon hours. Abundance of mosquitofish increased in the open water channels and among the *R. mangle* prop-root stands from morning hours (GLM, area vs. fish abundance:  $F_{1,176} = 170.3, p \leq 0.001$ ; time vs. fish abundance:  $F_{1,176} = 7.9, p \leq 0.01$ ; and area\*time vs. fish abundance:  $F_{1,176} = 1, p \geq 0.5$ ). Water temperatures in La Lunita remained the same in the open channels and between *R. mangle* prop-roots. Morning and afternoon water temperatures remained constant, only changing by an increase of  $0.3^{\circ}\text{C} \pm 0.9^{\circ}\text{C}$  (mean  $\pm$  SE) from morning to afternoon hours (GLM, area vs. temperature:  $F_{1,176} = 0, p \geq 0.05$ ; time vs. temperature:  $F_{1,176} = 79.7, p \leq 0.001$ ; and area\*time vs. temperature:  $F_{1,176} = 0, p \geq 0.05$ ).

### 3.2. Mosquitofish Behaviour

The open-water mosquitofish abundance in Soliman Bay significantly dropped across all three monitoring stations on six different sampling days. Areas further away from the strandline in Soliman Bay became warmer later in the day when compared to shallower waters closer to the strandline. Mosquitofish avoided the extreme water temperatures by swimming to the cooler areas of the open channels further out. This resulted in areas closer to

the strandline being less abundant of mosquitofish compared to areas further out from the strandline as time passed.

In the open channels of Soliman Bay mosquitofish abundance dropped by > 90% above an average water temperature of  $38.8^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$  (Fig. 5 A, mean  $\pm$  SE). Thus, when channel water temperatures exceeded  $38^{\circ}\text{C}$  during afternoon hours, a large shift in mosquitofish abundance was recorded, with  $44.1 \pm 2.8$  fish  $\text{m}^{-2}$  (mean  $\pm$  SE) before water temperatures reached critical limits reducing to  $10.4 \pm 1.1$  fish  $\text{m}^{-2}$  (mean  $\pm$  SE) after critical water temperatures were reached. Declines above the critical water temperature in open channels occurred at all stations on all days (ANOVA, temperature before and after critical thermal temperature vs. mosquitofish abundance in open water channels:  $F_{1,327} = 107.1$ ,  $p \leq 0.001$ ).

After 14:00 hrs mean water temperatures in the open water channel were almost  $10^{\circ}\text{C}$  hotter compared to morning water temperatures. Water temperatures were highest at the 50-metre station (GLM, time vs. temperature:  $F_{2,135} = 170.7$ ,  $p \leq 0.001$ ; distance from the strandline vs. temperature:  $F_{2,135} = 45.2$ ,  $p \leq 0.001$ ; and time\*distance from the strandline vs. temperature:  $F_{4,135} = 1.02$ ,  $p \geq 0.05$ ). All open-water Soliman Bay mangrove forest areas showed similar temperature increases between days. Mosquitofish abundance in the shade of the *Rhizophora* trees was significantly greater after open water reached critical temperatures (Fig. 5 B),  $35.1 \pm 2.6$  fish  $\text{m}^{-2}$  (mean  $\pm$  SE) compared with mosquitofish abundance in the shade of the trees before open water temperatures reached critical temperatures,  $13.3 \pm 2.5$  fish  $\text{m}^{-2}$  (mean  $\pm$  SE, ANOVA, temperature before and after critical thermal temperature vs. mosquitofish abundance in shaded areas:  $F_{1,52} = 28$ ,  $p \leq 0.001$ ). Burying behaviour and an increase of air-gulping behaviour by the mosquitofish in Soliman Bay was observed when water temperatures reached more than  $40^{\circ}\text{C}$ .

No large shifts of mosquitofish abundance in La Lunita was found in the open water channels throughout morning and afternoon hours. Mosquitofish abundance increased by ~20% from morning to afternoon hours within the open water channels (GLM, time vs. fish abundance:  $F_{1,84} = 4.8$ ,  $p < 0.05$ ; distance from the strandline vs. fish abundance:  $F_{2,8} = 1.6$ ,  $p \geq 0.05$ ; and time\*distance from the strandline vs. fish abundance:  $F_{2,84} = 1.5$ ,  $p \geq 0.05$ ). No difference of fish abundance was found between the three stations.

### 3.3. Parasol Shaded Area

Mosquitofish abundance was significantly higher in the open channels at Soliman Bay ( $17.2 \text{ fish} \pm 1.8 \text{ fish m}^{-2}$ , mean  $\pm$  SE) when compared to mosquitofish abundance under shading provided by parasols ( $1.9 \text{ fish} \pm 0.6 \text{ fish m}^{-2}$ , mean  $\pm$  SE) during afternoon hours (Fig. 6 A and B, Paired t-test:  $P \leq 0.001$ ,  $n = 30$ ). Water temperature in the open channels,  $38.6 \text{ }^{\circ}\text{C} \pm 0.1 \text{ }^{\circ}\text{C}$  (mean  $\pm$  SE) compared to water temperatures recorded in the parasol shaded areas during the same time,  $38.4 \text{ }^{\circ}\text{C} \pm 0.1 \text{ }^{\circ}\text{C}$  (mean  $\pm$  SE) were not significantly different ( $n = 30$ ). Water temperatures in the parasol shaded areas matched those of the ambient open water channel temperatures.

Salinity (psu) concentrations at Soliman Bay were  $11.5 \text{ psu} \pm 0.8 \text{ psu}$  (mean  $\pm$  SE) and were higher when compared with La Lunita,  $5.8 \text{ psu} \pm 0.2 \text{ psu}$  (mean  $\pm$  SE, Paired t-test:  $P \leq 0.001$ ,  $n = 10$ ). Dissolved  $\text{O}_2$  (mg/L) readings were significantly lower in the Soliman Bay water channels,  $5.4 \text{ mg/L} \pm 0.7 \text{ mg/L}$  (mean  $\pm$  SE) when compared with La Lunita water channels,  $11 \text{ mg/L} \pm 0 \text{ mg/L}$  (mean  $\pm$  SE, Paired t-test:  $P \leq 0.001$ ,  $n = 10$ ).

### 3.4. Food Availability

Zooplankton abundance in the open water channels was similar between both localities during the morning and afternoon hours (Fig. 7, two-way ANOVA, site\*time vs. zooplankton

abundance:  $F_{1,8} = 0.3$ ,  $p \geq 0.05$ ). During the morning in Soliman Bay there were  $301 \pm 33.9$  (mean  $\pm$  SE) zooplankton individuals per  $1 \text{ m}^3$ , and in the afternoon, there were  $415 \pm 65.9$  (mean  $\pm$  SE) zooplankton individuals per  $1 \text{ m}^3$ . In the La Lunita mangrove forest there were  $301 \pm 98.5$  (mean  $\pm$  SE) zooplankton individuals per  $1 \text{ m}^3$  in the morning compared with  $320.5 \pm 80.6$  individuals per  $1 \text{ m}^3$  in the afternoon.

### 3.5. Size distribution

Mosquitofish from Soliman Bay were on average  $> 10\%$  smaller (Fig. 8 A) when compared with mosquitofish from La Lunita, with  $18.7 \text{ mm} \pm 0.2 \text{ mm}$  (mean  $\pm$  SE) and  $21 \text{ mm} \pm 0.2 \text{ mm}$  (mean  $\pm$  SE) respectively (Paired t-test,  $P \leq 0.001$ ). A greater abundance of mature mosquitofish was found in La Lunita (Fig. 8 B). The largest mosquitofish measured in Soliman Bay was 36 mm, and in La Lunita the largest mosquitofish measured was 47 mm.

### 3.6. Predators

In Soliman Bay, large numbers of predatory birds were witnessed feeding on thermally-stressed mosquitofish showing a loss of equilibrium. During this time, water levels were low, and water temperatures were more than  $43^\circ\text{C}$  (Fig. 9 A and B).

## 4. DISCUSSION

Sites were not replicated during this study, so it was not possible to draw firm conclusions about site-specific environmental factors. But, our data reveal that when channel water was warmest in Soliman Bay, the mean water temperature shaded by the *R. mangle* trees was more than  $6^\circ\text{C}$  cooler - a significant temperature difference for mosquitofish, especially if reduced dissolved oxygen in the open water impacts feeding rates (Chippis & Wahl 2004), reduces growth rates (Wurtsbaugh & Cech 1983) and increases mortalities (Otto 1973). Water temperatures at the La Lunita mangrove forest was driven by daily flushing from inshore coastal water and the temperature remained constant throughout the day with little change and

1 with a maximum water temperature reaching 28°C. At times, water temperature in Soliman  
2 Bay was ~18°C hotter. Such an extreme water temperature will place very high physiological  
3 demands on the flora (Alongi 2018) and fauna.

4 The high abundance of mosquitofish foraging within open water channels at both  
5 localities during day time hours are consistent with fish behaviours observed from other studies  
6 (Ling & Willis 2005, Pyke 2005). However, dramatic declines in abundance of mosquitofish  
7 within the open channels at Soliman Bay during afternoon hours did not mirror the distribution  
8 patterns of mosquitofish observed in La Lunita during the same time. The high abundance of  
9 mosquitofish in Soliman Bay shifted from the open channels during morning hours to cooler  
10 *R. mangle* prop-root shaded areas in the afternoon. During the same time mosquitofish  
11 abundance in La Lunita remained the same in open water channels from morning to afternoon  
12 hours and no significant change in mosquitofish abundance was observed. The sharp rise in  
13 water temperature observed in Soliman Bay exceeded the critical thermal tolerance for the  
14 mosquitofish, which triggered their rapid migrations to seek environmental buffering.

15 Benoît et al. (2000) report that mosquitofish prefer habitats with lots of cover to avoid  
16 predation, but Casterlin & Reynolds (1977) report that mosquitofish do not favour such  
17 habitats. Our study highlights that mosquitofish behaviour is driven by water temperature and  
18 availability of a mangrove root refuge from predation. Our data are similar to Winkler (1979),  
19 who found that mosquitofish prefer water temperatures of 31 to 35°C, avoiding a critical water  
20 temperature limit of 39°C (Cherry et al. 1976) and an upper lethal limit of 38°C and above  
21 (Otto 1973). Despite the availability of many vegetated areas and complex habitats in Soliman  
22 Bay, mosquitofish only appeared to show preference to these habitats when water temperatures  
23 reached 38°C and above during afternoon hours. We found that mosquitofish in Soliman Bay  
24 preferred channel water temperature ranges of 35 to 37°C with a maximum thermal tolerance  
25 of 38 to 39°C, which agrees with previous studies of mosquitofish (Otto 1973, Cherry et al.



1976, Winkler 1979). Our results demonstrate that mosquitofish are actively seeking areas provided by *R. mangle* prop-roots when open channel water temperatures reach lethal limits of 38°C and above to avoid thermal stress. Prop roots also provide greater in-water complexity at the same time as the canopy provides shade. In addition, antipredator responses could also play a role as predatory birds were often observed.

Mosquitofish are opportunistic omnivores, consuming algae and zooplankton (Crivelli & Boy 1987). They are known to have extremely high feeding rates (up to 83 per cent of their total body weight per day) when exposed to water temperatures of 10°C to 35°C (Wurtsbaugh & Cech 1983, Chipps & Wahl 2004), very similar to the water temperatures at Soliman Bay from 10:00 to 12:30. Mosquitofish metabolic rates increase with temperature until DO<sub>2</sub> availability becomes too low (Cech et al. 1985). Thus, this may explain the high abundance of mosquitofish in the open channels at the Soliman Bay as the perfect feeding environment for mosquitofish would be between these times. Their appetites were corroborated by the fact that mosquitofish were observed in high abundance in open areas throughout the day continuously foraging for food, particularly on the water surface and occasionally on the surface of the sediment. The optimum feeding temperatures may also explain why mosquitofish abundance in the open water channels at La Lunita increased by almost 20 per cent during afternoon hours when water temperatures reached the ideal conditions for feeding. In addition, mosquitofish in Soliman Bay were not as numerous in the shade of the parasols compared to the open channels and shade of the mangrove trees. Unlike the mangrove prop roots, the parasols provided no additional underwater structure and did not provide any water temperature buffering.

Plankton tows from this study demonstrated that food availability remained constant throughout morning and afternoon hours at both localities and consisted of gastropods and crustaceans. High concentrations of zooplankton were found in all samples providing further

evidence that mosquitofish shifting abundance from open channels to cooler *R. mangle* shaded areas during afternoon hours at Soliman Bay were not driven by food availability but by avoiding extreme thermal stress. The lack of thermal stress and extreme water temperatures in La Lunita may explain why mosquitofish remained foraging in open channels throughout the day, as food was plentiful.

At birth mosquitofish are between 6 to 8 mm in length and grow at a rate of approximately 1 to 2 mm per week (Stearns 1983). This means that the greatest proportion of mosquitofish caught from both localities were aged between 3.5 and 6.5 weeks old. However, mosquitofish populations caught in La Lunita possessed higher ratios of fish aged 6.5 weeks and older. Male mosquitofish mature after 8 weeks and females mature after 10 weeks (Pyke 2005). This means that sexually mature individuals are more abundant in La Lunita. The size of mosquitofish caught in Soliman Bay were on average more than 10 per cent smaller than fish measured from La Lunita. Different predators and predation pressures coupled with extreme water temperatures in Soliman Bay may have caused changes with the *Gambusia* fish communities (Phenix et al. 2019). Stressful temperatures may have created developmental consequences for juvenile mosquitofish. Studies found that when tropical shallow-water fish are exposed to extreme water temperatures they exhibit energetic trade-offs to survive the heat stress, such as smaller body sizes, reduced energy reserves and increased rate of protein and lipid depletion (Madeira et al. 2017). Under stressful environments juvenile and vulnerable individuals divert more energy into dealing with stress instead of gonad and somatic development (Shahjahan et al. 2017, Wang et al. 2017). Such stress can also lead to higher mortalities (Madeira et al. 2017). Without cooler water provided by the *R. mangle* shade, populations of mosquitofish in Soliman Bay would suffer serious thermal stresses combined with increases of mortalities.

Improved fitness levels in mosquitofish are reported to occur at water temperatures of 30°C with female mosquitofish investing higher proportions of energy into reproduction at average water temperatures of 25°C (Wurtsbaugh & Cech 1983). A similar water temperature environment was found in La Lunita and may explain why the abundance and size distribution of mosquitofish were greater at this locality. Mosquitofish growth can be reduced by lower food availability and by water temperatures above 35°C (Wurtsbaugh & Cech 1983). Due to the extreme water temperatures at Soliman Bay mosquitofish sacrifice feeding in open water channels in favour of reduced environmental stressors provided by the *R. mangle* stands.

Air-gulping behaviour expressed by mosquitofish was observed in Soliman Bay. This behaviour was previously documented with fish known to survive in DO<sub>2</sub> concentrations as low 0.28 mg/l, providing they can breathe atmospheric air from surface waters (Pyke 2005). As dissolved oxygen in the general water column approaches zero, mosquitofish survival depends on their ability to gain access to the surface water (Homski et al. 1994). They either take in water that is relatively oxygen-rich at the atmosphere-water interface (Lewis 1970) or gulp air from the atmosphere. Their dorsally oriented mouth and dorso-ventrally flattened head is the ideal morphology for breathing at the surface-water interface (Lewis 1970).

The mosquitofish burying behaviour observed in this study has not previously been reported. Further studies would be needed to confirm this novel behaviour, as our study indicates that mosquitofish are also utilising cooler temperatures provided within sediments shaded by *R. mangle* trees.

## 5. Conclusion

Ecosystem services derived from mangrove roots in the form of the nursery function have been well-documented (Beck et al. 2001, Nagelkerken 2009, Alongi 2014). However, the role of environmental buffering is less understood. This study gives an example of how *R. mangle*

prop-roots and canopy may reduce thermal stress, provide environmental buffering and protection for animals in shallow, stagnant water in a Caribbean mangrove forest. The ecological function of thermal buffering may also benefit many other mangrove fauna (Hendy et al. 2013 & 2014). The importance of mangrove hydrology and subsequent maintenance of thermoregulation (Teutli-Hernández & Herrera-Silveira 2018) is also highlighted in this study. Water temperatures in La Lunita remained at optimum levels for mosquitofish fitness and reproduction (Wurtsbaugh & Cech 1983). In Soliman Bay however, thermal extremes impacted mosquitofish ecology, which may include impacts on their size and feeding activity. This study highlights the importance of environmental buffering provided by the *R. mangle* stands. Conservation and protection of these important ecosystems will serve to maintain fish populations in a changing climate. Biodiversity maintenance offered by mangrove forests to juvenile and vulnerable marine animals demonstrates the need for their protection and restoration to maintain ecosystem-level biomass and productivity. Management planning of mangrove ecosystems, especially where major harvesting is involved should take extreme care to ensure that *Rhizophora* stands are retained within the ecosystem to protect important ecological functions.

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## LITERATURE CITED

Alongi MD (2002) Present state and future of the world's mangrove forests. *Environ Conserv* 29: 331–349

- 1 Alongi MD (2014) Carbon Cycling and Storage in Mangrove Forests. *Annu Rev Mar Sci*  
2 6:195–219
- 3 Alongi MD (2018) Impact of global change on nutrient dynamics in mangrove forests.  
4 *Forests* 9: 596
- 5 Aral MM, Guan J (2016) Global sea surface temperature and sea level rise estimation with  
6 optimal historical time lag data. *Water* 8: 519
- 7 Baird SF, Girard C (1853) Descriptions of new species of fishes collected by Mr. John H.  
8 Clark, on the U.S. and Mexican boundary survey, under Lt. Col. Jas. D. Graham.  
9 *Proc. Acad. Nat. Sci.* 6: 387–390
- 10 Beck MW, Heck KL, Able KH, Childers DL, Eggleston DB, Gillanders BM, Halpern B,  
11 Hays CG, Hoshion K, Minello TJ (2001) The identification, conservation, and  
12 management of estuarine and marine nurseries for fish and invertebrates: A better  
13 understanding of the habitats that serve as nurseries for marine species and the  
14 factors that create site-specific variability in nursery quality will improve  
15 conservation and management of these areas. *BioScience* 51: 633–641
- 16 Benoit HP, Post JR, Barbet AD (2000) Recruitment dynamics and size structure in  
17 experimental populations of the mosquitofish, *Gambusia affinis*. *Copeia* 2000: 216-  
18 221
- 19 Casterlin ME, Reynolds WW (1977) Aspects of habitat selection in the mosquitofish  
20 *Gambusia affinis*. *Hydrobiologia* 55: 125-127
- 21 Cech JJ, Massingill MJ, Vondracek B, Linden AL (1985) Respiratory metabolism of  
22 mosquitofish, *Gambusia affinis*: effects of temperature, dissolved oxygen, and sex  
23 difference. *Environ Biol Fish* 13: 297-307
- 24 Cherry DS, Guthrie RK, Rodgers JH, Cairns J, Dickson KL (1976) Responses of  
25 mosquitofish (*G. affinis*) to ash effluent and thermal stress. *Trans Amer Fish Soc*  
26 105: 686–694
- 27 Chipps SR, Wahl DH (2004) Development and evaluation of a Western mosquitofish  
28 bioenergetics model. *Trans Amer Fish Soc* 133: 1150-1162

- Crivelli AJ, Boy V (1987) The diet of the mosquitofish *Gambusia affinis* (Baird and Girard) (Poeciliidae) in Mediterranean France. *Revue d'écologie – la terre et la vie* 42: 421-435
- Donato DC, Kauffman JB, Murdiyarso D, Kurnianto S, Stidham M, Kanninen M (2011) Mangroves among the most carbon-rich forests in the tropics. *Nat Geosci* 4: 293-297
- Doney SC, Ruckelshaus M, Duffy E, Barry JP, Chan F, English CA, Galindo HM, Grebmeier JM, Hollowed AB, Knowlton N, Polovina J, Rabalais NN, Sydeman WJ, Talley LD (2011) Climate Change Impacts on Marine Ecosystems. *Annu Rev Mar Sci* 4:1–4
- Duke NC, Meynecke JO, Dittmann S, Ellison AM, Anger K, Berger U, Cannicci S, Dielle K, Ewel KC, Field CD, Koedam N, Lee SY, Marchand C, Nordhaus I, Dahdouh-Guebas F (2007) A World without mangroves? *Science* 317: 41–42
- Duke NC, Schmitt K (2015) Mangroves: unusual forests at the seas edge. *Trop For Handbook* DOI 10.1007/978-3-642-41554-8\_129-1
- Eddy S, Ridho MR, Iskandar I, Mulyana A (2016) Community based mangrove forests conservation for sustainable fisheries. *J Trop Silv* 7: 42-47
- Gabriel JJ, Reinhardt EG, Peros MC, Davidson DE, Hengstum PJ, Beddows PA (2008) Palaeoenvironmental evolution of Cenote Aktun Ha (Carwash) on the Yucatan Peninsula, Mexico and its response to Holocene sea-level rise. *J Paleolimnol* 42: 199–213
- Hendy IW, Eme J, Dabruzzi TF, Nembhard RV, Cragg SM, Bennett WA (2013) Dartfish use teredinid tunnels in fallen mangrove wood as a low-tide refuge. *Mar Ecol Prog Ser* 486: 237-245
- Hendy IW, Michie M, Taylor BW (2014) Habitat creation and biodiversity maintenance in mangrove forests: teredinid bivalves as ecosystem engineers. *PeerJ* 2:e591
- Homski D, Goren M, Gasith A (1994) Comparative evaluation of the larvivorous fish *Gambusia affinis* and *Aphanius dispar* as mosquito control agents. *Hydrobiologia* 284: 137–146

- Hupfeld RN, Phelps QE, Flammang MK, Whitledge GW (2015) Assessment of the effects of high summer water temperatures on shovelnose sturgeon and potential implications of climate change. *River Res. Applic* 31: 1195-1201
- Hurlbert SH, Mulla MS (1981) Impacts of mosquitofish (*Gambusia affinis*) predation on plankton communities. *Hydrobiologia* 83: 125-151
- Hutchison J, Manica A, Swetnam R, Balmford A, Spalding M (2013) Predicting Global Patterns in Mangrove Forest Biomass. *Conserv Lett* 7: 233–240
- IPCC. 2014. In *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Pachauri, R. K. & Meyer, L. A.) 2–34
- Ismail H, Abd AK, Alias NE (2012) Determination of mangrove forest performance in reducing tsunami run-up using physical models. *Nat Hazards* 63: 939-963
- Jeffries KM, Cannon RE, Davis BE, Komoroske LM, Britton MT, Sommer T, Todgham AE, Fangue NA (2016) Effects of high temperatures on threatened estuarine fishes during periods of extreme drought. *J Exp Biol* 219: 1705-1716
- Laegdsgaard P, Johnson C (2001) Why do juvenile fish utilize mangrove habitats? *J Exp Mar Biol Ecol* 257: 229–253
- Lee SY, Primavera JH, Dahdouh-Guebas F, McKee K, Bosire JO, Cannicci S, Diele K, Fromard F, Koedam N, Marchand C, Mendelssohn I, Mukherjee N, Record S (2014) Ecological role and services of tropical mangrove ecosystems: a reassessment. *Global Ecol Biogeogr* 23: 726-743
- Lee F, Simon KS, Perry GLW (2017) Increasing agricultural land use is associated with the spread of an invasive fish (*Gambusia affinis*). *Sci Tot Env* 586: 1113–1123
- Lewis WM (1970) Morphological adaptations of cyprinodontoids for inhabiting oxygen deficient waters. *Copeia* 1970: 319–326
- Lewis RR, Milbrandt EC, Brown B, Krauss KW, Rovaie A, Beever JW, Flynn LL (2016) Stress in mangrove forests: Early detection and pre-emptive rehabilitation are essential for future successful worldwide mangrove forest management. *Mar Poll Bull* 109: 764-771

- Ling N, Willis K (2005) Impacts of mosquitofish, *Gambusia affinis*, on black mudfish, *Neochanna diversus*. New Zea J Mar Freshw Res 39:1215-1223
- Luck GW, Daily GC, Ehrlich PR (2003) Population diversity and ecosystem services. *Tren Ecol Evo* 18: 331-336
- Madeira D, Narciso L, Cabral HN, Vinagre C, Diniz MS (2013) Influence of temperature in thermal and oxidative stress responses in estuarine fish. *Comp Biochem Physiol A* 166: 237-243
- Maulvault AL, Barbosa V, Alves R, Custodio A, Repolho PAT, Ferreira PP, Rosa R, Marques A, Diniz M (2017) Ecophysiological responses of juvenile seabass (*Dicentrarchus labrax*) exposed to increased temperature and dietary methylmercury. *Sci Total Environ* 586: 551-558
- Munday PL, Donelson JM, Domingos JA (2017) Potential for Adaption to Climate Change in Coral Reef Fish. *Glob Change Biol* 23: 307–317
- Muzaki FK, Giffari A, Saptarini D (2017) Community structure of fish larvae in mangroves with different root types in Labuhan coastal area, Sepulu – Madura. *AIP Conf Proc.* 1854: 020025-1–020025-6; doi: 10.1063/1.4985416
- Nagelkerken, I (2009) *Evolution of Nursery Function of Mangroves and Seagrass Beds for Tropical Decapods and Reef Fishes: Patterns and Underlying Mechanisms*. Dordrecht: Springer
- Otto RG (1973) Temperature tolerance of the mosquitofish, *Gambusia affinis* (Baird and Girard). *J Fish Biol* 5: 575–585
- Pershing AJ, Alexander MA, Hernandez CM, Kerr LA, Bris A, Mills KE, Nye JA, Record NR, Scannell HA, Scott JD, Sherwood GD, Thomas AC (2015) Slow adaptation in the face of rapid warming leads to collapse of the Gulf of Maine cod fishery. *Sci* 350: 809-812
- Phenix LM, Tricarico D, Quintero E, Bond ME, Brandl SJ, Gallagher AJ (2019) Evaluating the effects of large marine predators on mobile prey behavior across subtropical reef ecosystems. *Ecol Evol* 1-12
- Pinsky ML, Eikeset AM, McCauley DJ, Payne JL, Sunday JM (2019) Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature* 569: 108



- Pörtner HO, Knust R (2007) Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* 315: 95 DOI: 10.1126/science.1135471
- Pyke GH (2005) A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Rev Fish Biol Fisher* 15: 339-365
- Rog SM, Clarke RH, Cook CN (2016) More than marine: revealing the critical importance of mangrove ecosystems for terrestrial vertebrates. *Diversity Distrib* 23: 221-230
- Scholander PF, van Dam L, Scholander SI (1955) Gas exchange in the roots of mangroves. *Am J Bot* 92-98
- Shahjahan MD, Kitahasi T, Ando H (2017) Temperature affects sexual maturation through the control of kisspeptin, kisspeptin receptor, GnRH and GTH subunit gene expression in the grass puffer during the spawning season. *Gen Comp Endocr* 243: 138-145
- Stearns SC (1983) The evolution of life-history traits in mosquitofish since their introduction to Hawaii in 1905: rates in evolution, heritabilities, and developmental plasticity. *Amer Zool* 23: 65-75
- Teutli-Hernández C, Herrera-Silveira JA (2018) The Success of Hydrological Rehabilitation in Mangrove Wetlands Using Box Culverts Across Coastal Roads in Northern Yucatán (SE, México). *Spring Nat* 25:607-619
- Wang X, Liu Q, Xiao Y, Yang Y, Wang Y, Song Z, You F, An H, Li J (2017) High temperature causes masculinization of genetically female olive flounder (*Paralichthys olivaceus*) accompanied by primordial germ cell proliferation detention. *Aquaculture* 479: 808-816
- Wiens JJ (2016) Climate-related local extinctions are already widespread among plant and animal species. *PLoS biol* 14: e2001104
- Winkler P (1979) Thermal preference of *Gambusia affinis affinis* as determined under field and Laboratory conditions. *Copeia* 1979: 60-64
- Wurtsbaugh WA, Cech J (1983) Growth and activity of juvenile mosquitofish: temperature and ration effects. *Trans Am Fish Soc* 112: 653-660

## Figures

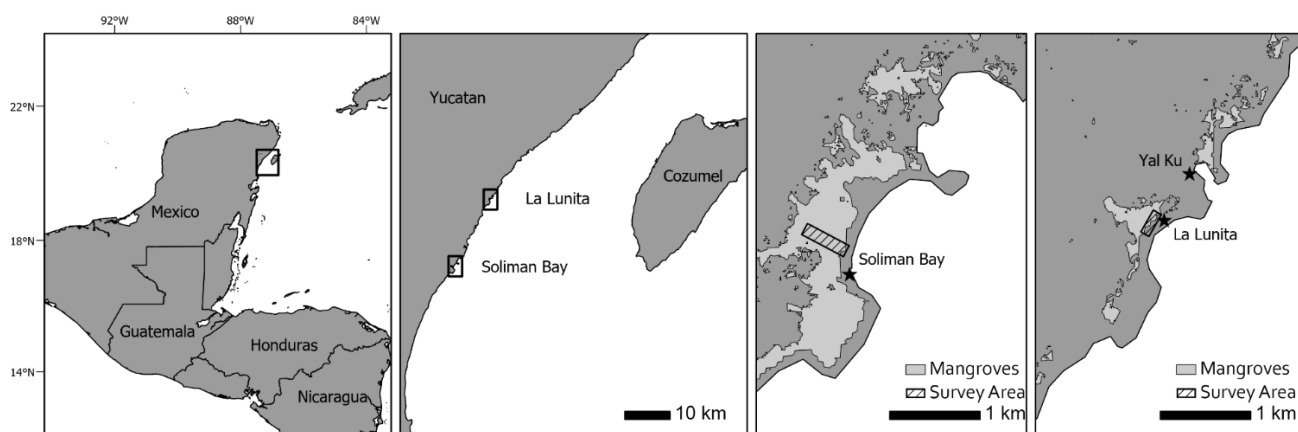


Fig. 1. Map illustrating country, region and site-specific scale mangrove localities. The area surveyed within each mangrove forest is highlighted.

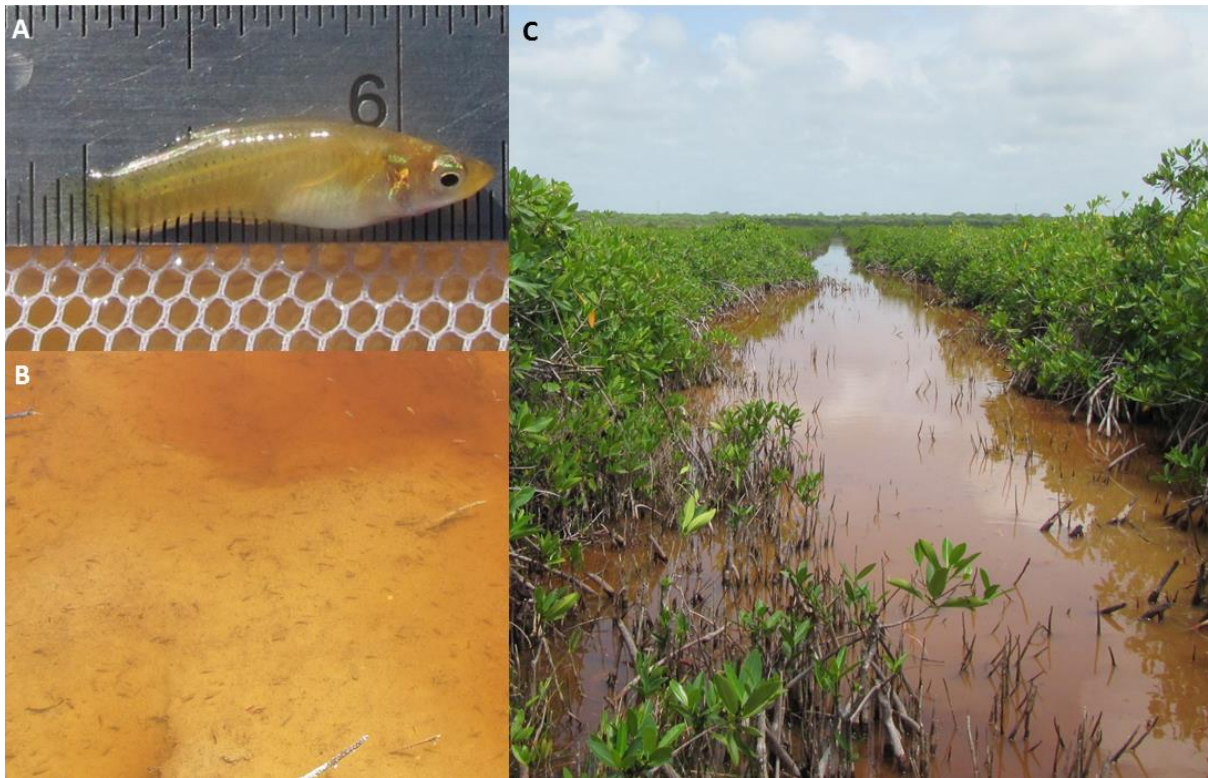


Fig. 2. Soliman Bay mangrove forest, Tulum, Mexico: (A) an adult female mosquitofish. (B) Dense shoals of mosquitofish foraging for food in open channels and (C) The main channel in the Soliman Bay mangrove forest where large numbers of mosquitofish forage for food in the morning.

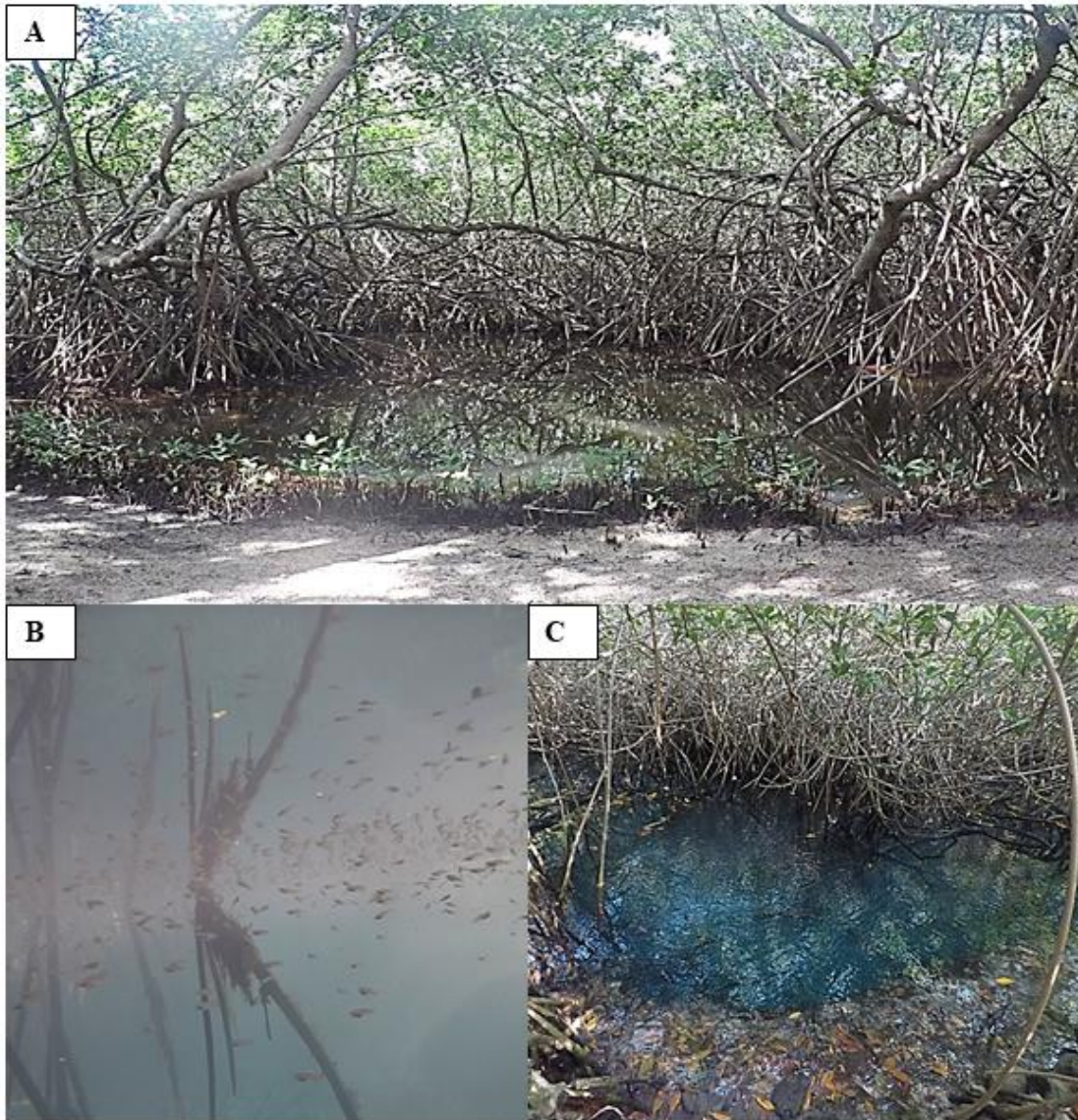


Fig. 3. The La Lunita mangrove forest, Tulum, Mexico: (A) The strand line channel lined with large *R. mangle* trees. (B) Dense shoals of large mosquitofish foraging for food in open channels and *Rhizophora* roots (C) One of many cenote openings with deep flowing water.



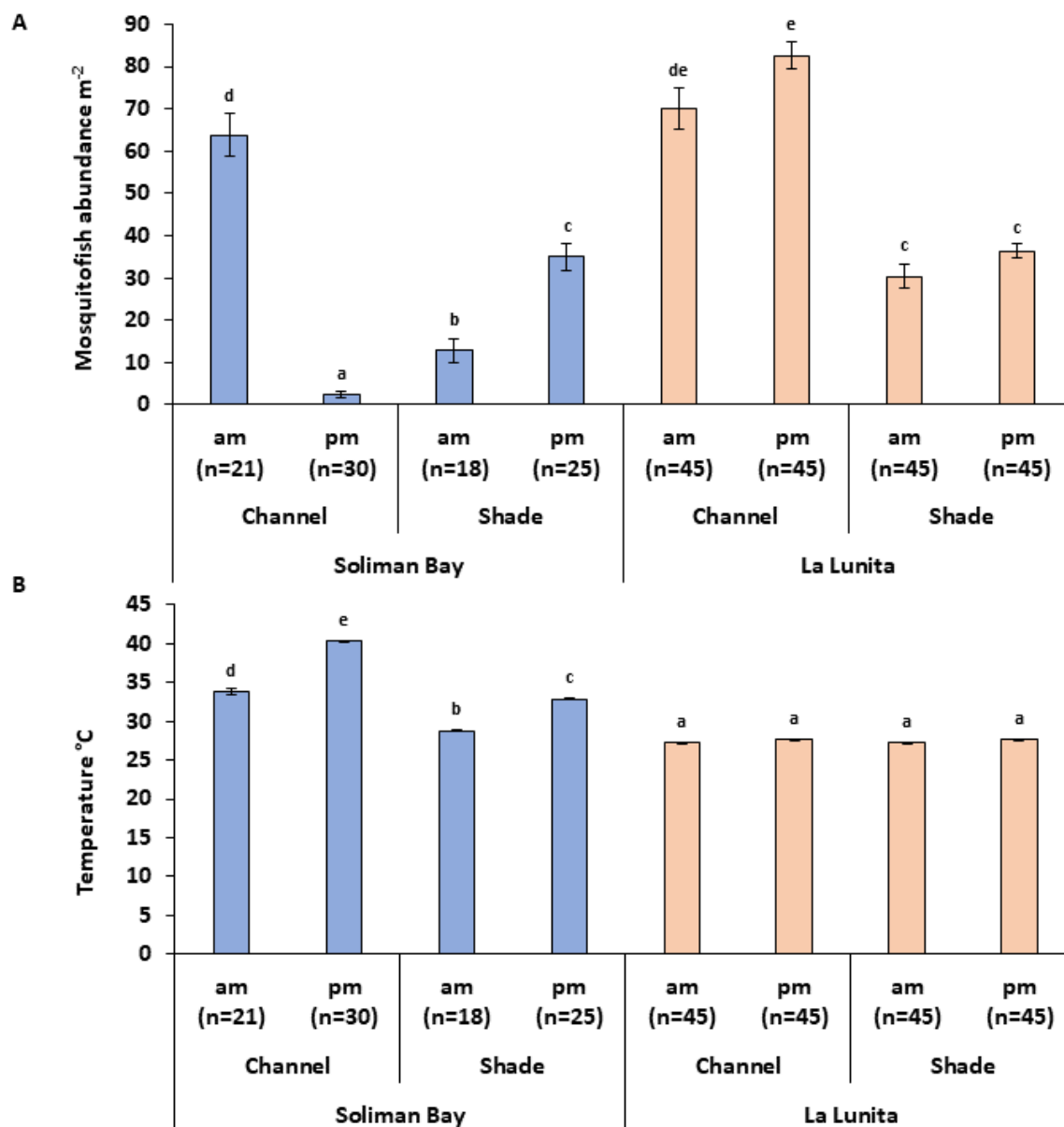


Fig. 4: Temporal and spatial variations of mosquitofish abundance and water temperature in open channels and prop root shaded areas in the Soliman Bay and La Lunita localities: (A) mosquitofish abundances (individuals m<sup>-2</sup>, mean  $\pm$  SE) during morning (am) and afternoon (pm) hours in open water channels and shaded *R. mangle* prop-root areas. GLM, area (channel & roots) and time (am & pm) vs. fish abundance: area vs. fish abundance:  $F_{1,87} = 8.5$ ,  $p \leq 0.01$ ; time vs. fish abundance:  $F_{1,87} = 39.1$ ,  $p \leq 0.001$ ; and area\*time vs. fish abundance:  $F_{1,87} = 177.2$ ,  $p \leq 0.001$  (B) temperature measurements (°C, mean  $\pm$  SE) during morning (am) and afternoon (pm) hours in open water channels and shaded *R. mangle* prop-root areas. GLM, area vs. temperature:  $F_{1,87} = 799.2$ ,  $p \leq 0.001$ ; time vs. temperature:  $F_{1,87} = 606.2$ ,  $p \leq 0.001$ ; and area\*time vs. temperature:  $F_{1,87} = 28.5$ ,  $p \leq 0.001$ ). Letters above the bars = Tukey's pairwise comparisons tests,  $n$ -values = number of quadrats.

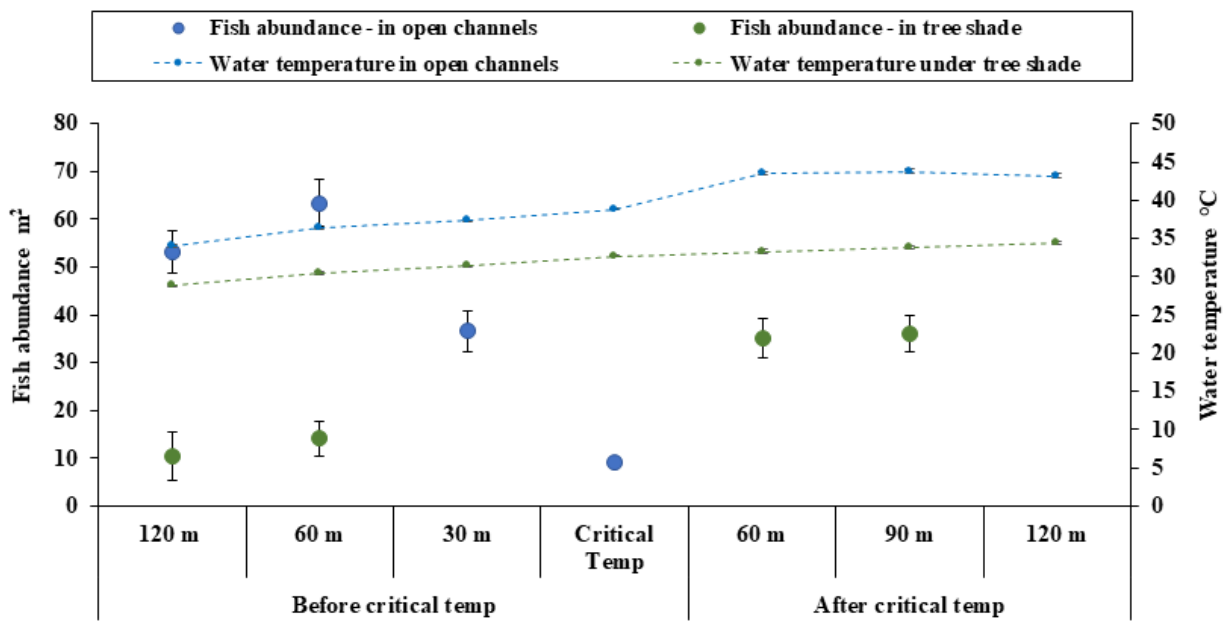


Fig. 5: The abundance of mosquitofish (individuals  $m^{-2}$ ) and water temperatures estimated in open channels and among tree shade areas in Soliman Bay (mean  $\pm$  SE), recorded 120 minutes before and after critical temperatures were reached.

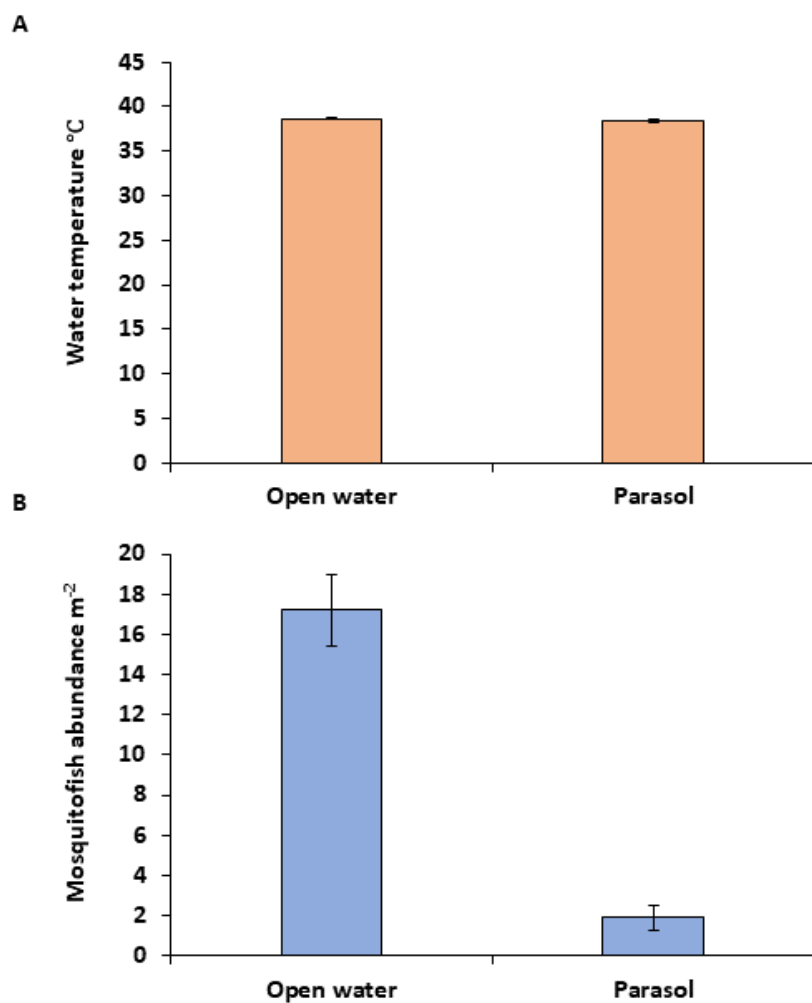


Fig. 6. A) Water temperatures and B) distributions of mosquitofish (Paired t-test:  $P \geq 0.05$  and  $P \leq 0.001$ , respectively, mean  $\pm$  SE) in open water and in shaded parasol areas in Soliman Bay observed during afternoon hours.

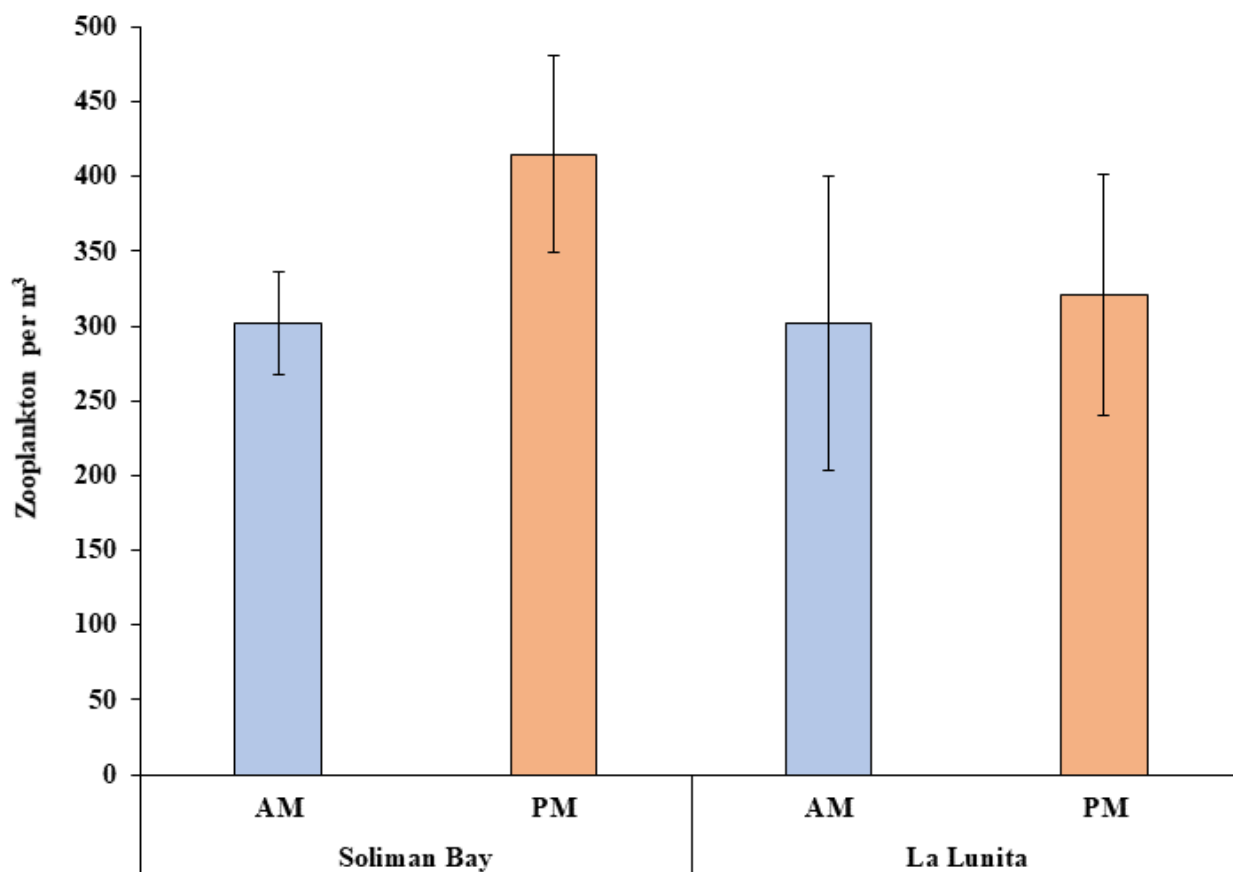


Fig. 7. Zooplankton abundance estimated from 6 plankton tows in the Soliman Bay and La Lunita mangrove forests (mean  $\pm$  SE, two-way ANOVA,  $P \geq 0.05$ ).



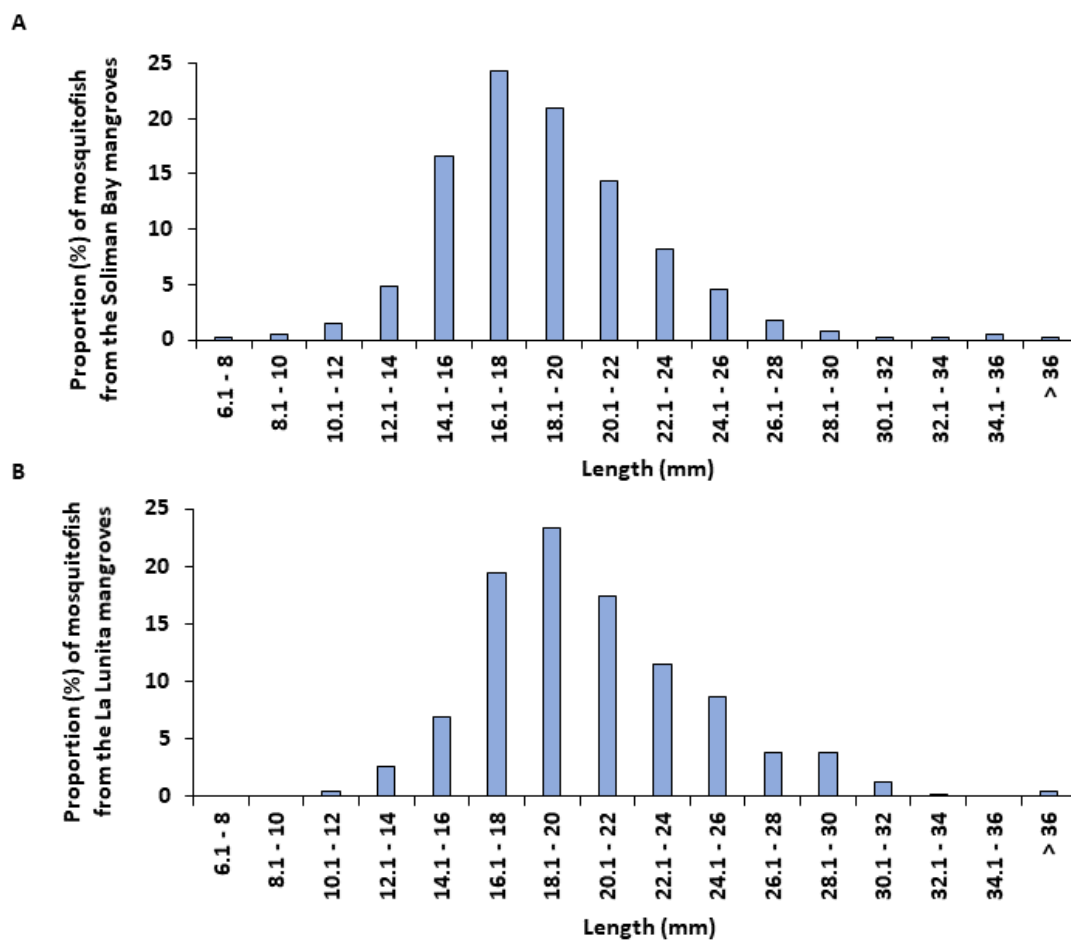


Fig. 8: Estimated lengths (mm) of mosquitofish caught from four nettings within the, (A) Soliman Bay ( $n = 391$ ), and (B) five nettings from La Lunita ( $n = 391$ ) mangrove forests.



Fig. 9. A. Egrets feeding on thermally stressed mosquitofish, B. An attacked and dead mosquitofish, and C. Highlighted in red, mosquitofish burying into sediment when channel water temperatures reached more than 43 °C during the afternoon in the Soliman Bay mangrove forest.